Cell growth characteristics of equine synovial fluid stem cells

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Abstract

Equine mesenchymal stem cells (MSCs) have been isolated from various sources such as: peripheral blood, bone marrow, adipose tissue, umbilical cord, placenta, Wharton Jelly. Besides these synovial fluid and synovial membrane represents a promising source of mesenchymal stem cells, which can be harvested by minimally invasive methods. MSCs from these sources have the potential to self-renew and differentiate into multiple lineages such as chondrogenic, adipogenic and osteogenic. The aim of our study was to evaluate the growth characteristics of equine synovial liquid stem cells harvested from the tarsometatarsal joint during arthroscopic surgery. Samples were collected in a sterile syringe and were diluted and centrifuged at 1500rpm for 7 min. The supernatant were removed and the cells were resuspended in propagation medium: Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% antibiotic-antimycotic (Sigma-Aldrich). The medium was changed after 3 days. The proliferation ability, cell doubling number, cell doubling time, daily duplication rate and clonogenic efficacy was evaluated. Isolated cells exhibited plastic adherence capacity, monolayer growth, and fibroblast-like morphology, high growth and clonogenic capacity. Our study demonstrated the characteristics of equine synovial fluid derived stem cells, an ideal candidate for veterinary regenerative medicine.

Keywords: *stem cells, synovial fluid, horse, proliferation, cells growth*

Introduction

Mesenchymal stem cells (MSCs) are multipotent precursor cells with self-renewal and differentiation capacity (Dominici et al. 2006, Pall et al., 2015). MSCs are a promising therapeutic tool in veterinary medicine (Bahamondes et al., 2017) have been isolated from different animal species including rodents (Penny et al., 2012), dogs (Guercio et al., 2013, Bahamondes et al., 2017), horses (Barberini et al., 2014, Pall et al., 2016) and rabbits (Tan et al., 2013). According to the International Society for Cellular Therapy published in 2006, human MSCs are characterized by plastic adherence capacity, trilineage differentiation capacity and expression of surface markers such as CD105, CD90, CD44, CD73, CD79α and are negative for the expression of hematopoietic markers CD34, CD45 and CD19, CD14, human leukocyte antigen HLA-DR (Dominici et al., 2006). MSCs isolated from animals are not fully characterized. Equine mesenchymal stem cells (MSCs) have been isolated from various sources such as: peripheral blood, bone marrow, adipose tissue, umbilical cord, placenta, Wharton Jelly (Barberini et al., 2014, Tessier et al., 2015, Pall et al., 2016). Besides these synovial fluid and synovial membrane represents a promising source of mesenchymal stem cells, which can be harvested by minimally invasive methods. The aim of our study was to evaluate the growth characteristics of equine synovial liquid stem cells harvested from the tarsometatarsal joint during arthroscopic surgery.

Material and methods

Samples (n=5) were collected in a sterile syringe during atroscopic surgery. The samples were harvested after owner's agreement. The samples were diluted and centrifuged at 1500rpm for

7 min. The supernatant were removed and the cells were resuspended in propagation medium: Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% antibiotic-antimycotic (Sigma-Aldrich). Cultures were incubated at 37 °C in humidified atmosphere with 5 % CO_2. After 72 h, non-adherent cells were removed and the medium was replaced.

Proliferation ability of equine synovial fluid derived stem cells was determined using MTT colorimetric assay. A concentration of $1x10^4$cells/well was cultured in a 96-well plate. After 24 h 20 µl MTT (2 mg/ml, Sigma-Aldrich) was added to each well and cultures were incubated at 37 °C for 3 h. The fromazan were dissolved with 100 µl DMSO (dimethylsulfoxide, Sigma-Aldrich) and the absorbance was measured at 450 nm using a spectrophotometer (Bio-Rad, Hercules, CA, USA). In order to evaluate the proliferation rate the population doubling time were assessed. A total number of $1x10^5$ cells/ well were seeded in 24-well cell culture plates. After 24 h (t24h) the non-adherent and the adherent cells (N0) were counted. 24 h later (t48h) the adherent cells from three wells were counted (N48h). The doubling time (tD) was calculated according to the formula: $tD = (\log 2 \times t) / (\log N48h - \log N0)$.

The clonal capacity of cells was evaluated using CFU-F assay. $5x10^2$cells/cm^2 was cultured in expansion medium. After 10 days the cells were fixed with 4% paraformaldehyde for 10 min and stained with 0.5% crystal violet (Sigma-Aldrich, St. Louis, MO) in 10% methanol for 20 minutes and were examined under phase contrast inverted light microscope (Nikon TS100, Nikon Instruments, Europe) and the colonies (> 50 cells) were counted. CFU-F efficiency was estimated according to the formula: CFU-F efficiency = (counted CFU-F/cells originally seeded) × 100.

Results and discussion

MSCs were isolated from harvested from equine tarsometatarsal joint during arthroscopic surgery. The isolated cells were characterized to confirm their caracteristics such as plastic adherence, expression of specific surface antigens and differentiation potential. Equine synovial fluid derived mesenchymal stem cells showed fibroblast-like morphology (fig 1.), adherence to plastic surface and express the specific surface markers (CD90, CD105, CD44, results not shown here). The proliferation capacities of cells were evaluated using MTT assay.

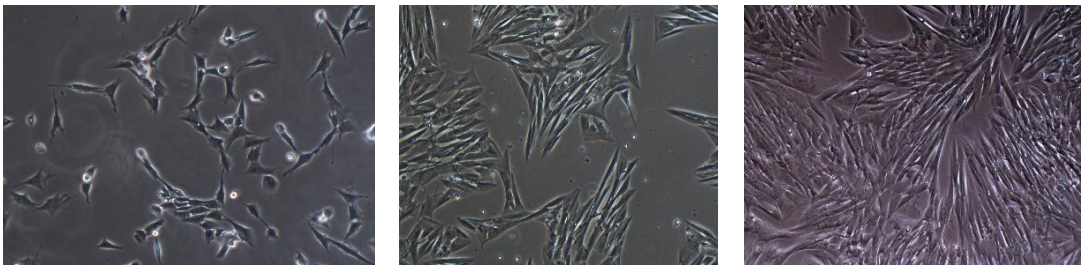

Figura 1. Equine synovial fluid derived cell morphology

The isolated cells lines exhibited similar proliferation potential in evaluated period (d1-D6). Optical density (*OD*) values at each time were evaluated (fig 2.).

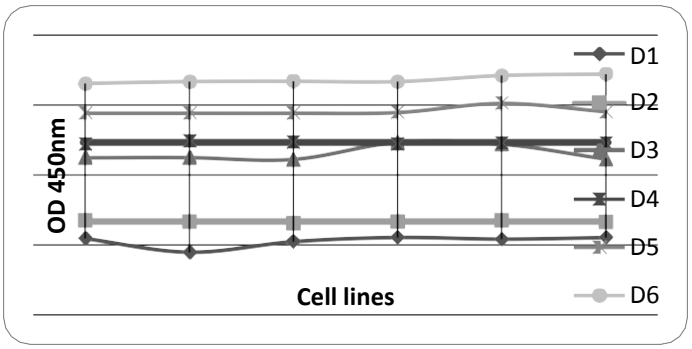

Figure 2. Proliferation potencial of equine synovial fluid derived mesenchymal stem cell

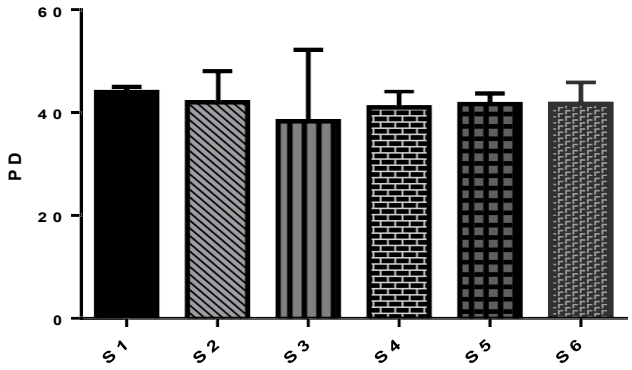

Figure 3. Population doubling time in equine synovial fluid derived mesenchymal stem cell lines

Individual assessment at each cell line can be concluded that for line S1 the population doubling time was 44±1.01h, 42±5.8 in S2, 38.33±13.86 h in S3, 41.00±3.00 in S4, 41.66±2.08 in S5 and 41.66±4.16 in S6 (fig. 3). The average of population-doubling time (PD) was 41.44 ± 1.83 h.

The clonogenic potential of MSCs was assessed by CFU-F assay. Isolated cells lines displayed colony-forming ability; the frequency of colony forming cells for S1 was 37.66±6.42 colonies/100 cm2, 36.66±6.42 colonies/100 cm^2 in S2, 36.33±7.57 colonies/100cm_2 in S3, 38.66±4.04 colonies/100cm2 in S4, 35±4.35 in S5 and 37±4.58 colonies /100cm2 in S6 (fig 4.).

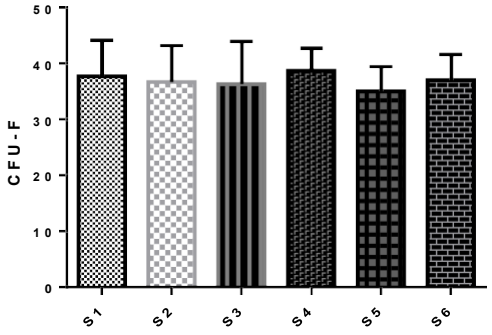

Figure 4. The average of colonigenic potential (CFU-F assay) of equine synovial fluid derived mesenchymal stem cell

Multipotent mesenchymal stem cells (MSCs) represent a promising source of cells for regenerative medicine therapeutic approaches in both human and veterinary medicine given their properties (Singer et al., 2011, Carrade et al., 2013, Clark et al., 2016, Pall et al., 2016). MSCs can be defined by their morphology, expression of a panel of cell surface markers and their tri-lineage differentiation (Dominici et al., 2006, Clark et al., 2016). In our study, cells from synovial fluid were evaluated as possible sources of MSCs, for equine regenerative medicine. The horse is a valuable species for evaluating the usefulness and efficiency of MSC treatment (Pezzanite et al., 2015). MSCs from different sources are used, but most often autologous bone marrow-derived MSCs are used to treat musculoskeletal disorders including tendonitis, osteoarthritis, cartilage damage, and meniscal injuries (Schnabel et al., 2009, Frisbie and Smith 2010, Caniglia et al., 2012, De Schauwer et al., 2013, Pezzanite et al., 2015).

MSCs also can modulate endogenous tissue and immune cells (Parekkadan et al., 2010) and can migrate to the site of injury after receiving specific signals (Chen et al., 2011). MSCs also can be subject to cryopreservation with minimal loss of potency (Lee et al., 2005), for future heterologous or autologous transplantation. Numerous studies and clinical trials have demonstrated the role of MSCs, but further studies are needed to elucidate the exact therapeutic mechanisms.

Conclusions

Our study showed that mesenchymal stem cells could be successfully isolated form synovial fluid. Characterization of these cells can be achieved based by their morphology, immunophenotype and differentiation potential. Results from our study demonstrate the proliferative and clonogenic potential of equine synovial fluid derived stem cells, and can serve as a potential source of mesenchymal stem cells for veterinary regenerative medicine.

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